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Anti quorum sensing activities of medicinal plant extracts against quorum sensing mediated virulence factors of *Pseudomonas aeruginosa*

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ABSTRACT

Quorum sensing (QS) a cell density-dependent regulation of virulent bacterial gene expression which regulates pathogenesis of bacterial infection. QS is an effective strategy for the development of novel antimicrobial agents which can overcome antibiotic resistance. In the present study, anti quorum sensing activities of ethanolic extracts of medicinal plants Curcuma longa, Ocium tenuiflorum, Aegle marmelos, Eucalyptus globules, Azadirachta indica and Cynodon dactylon against quorum sensing mediated virulence factors such as twitching motility, biofilm production, pyocyanin production and proteolytic activity of Pseudomonas aeruginosa has been carried out. Among the plants Eucalyptus globules revealed maximum inhibition of QS mediated virulence factors. Twisting motility, biofilm formation and total protease activity have been shown to inhibited at maximum level by Eucalyptus globules. Cynodon dactylon showed enhanced cell adhesion inhibition. Maximum reduction of pyocyanin production was recorded in Aegle marmelos. The present study revealed potential of these plant extracts in treating microbial infections through cell growth inhibition or quorum sensing inhibitors would suggests the possible utilization for the prevention of bacterial infections.

Key words: Quorum Sensing, Plant Extracts, Virulrnce Factors, *Pseudomonas Aeruginosa*

INTRODUCTION

Control of microbial infections by inhibition of microbial growth or quorum sensing has been the base of antimicrobial chemotherapy. However, an emerging problem associated with misuse of antibiotic therapy is the worldwide emergence of higher level tolerance of target organisms against available broad spectrum antibiotics [1]. As a result, and in the light of the rapid spread of multidrug resistance, the development of new antimicrobial or antipathogenic agents that act upon new microbial targets has become a very pressing priority [2]. In view of the fact that quorum sensing is involved in microbial pathogenesis, research efforts have focused recently upon developing antipathogenic agents to control bacterial diseases by inhibiting quorum sensing [3,4]

Quorum sensing (QS) is a process of cell to cell communication that allows bacteria to share information about cell density and control the gene expression accordingly. Among the many traits controlled by quorum sensing, the expression of virulence factors by pathogenic bacteria in the quorum-sensing like *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Vibrio cholera*. Quorum sensing process that involves the production, detection, and response to extracellular signalling molecules called auto inducers. These auto inducers accumulate in the surrounding environment and in the presence of a large population of cells, the concentration accumulates to a level needed for virulence(.Quorum sensing is thought to afford pathogenic bacteria a mechanism to minimize host immune responses by delaying the production of tissue-damaging virulence factors until sufficient bacteria have amassed and are prepared to overwhelm host defence mechanisms and establish infection [5].

Pseudomonas aeruginosa is a Gram-negative bacterium capable of surviving in a wide range of environments. This organism is an opportunistic pathogen and it is commonly associated with nosocomial infections and infections of severely burned individuals, and is a leading cause of death in severe respiratory infections, such as chronic lung infections in CF patients Infections with *P. aeruginosa* are difficult to eradicate, due to their high levels of antibiotic resistance and growth in biofilms due to the production of multiple virulence factors [6,7]. Quorum sensing system is responsible for inducing pathogenicity by producing various virulence factors as twisting motility, biofilm formation and cell adhesion, pyocyanin production, proteolytic enzyme activity.

Twitching motility is a flagellum-independent mode of surface translocation which requires functional type 4 pili and clearly associated with high cell densities and cell-cell communication, which are the hallmarks of quorum-sensing systems in bacteria. The role of auto inducer-mediated signalling, or quorum sensing, in the control and expression of twitching motility which required for adherence to human bronchial epithelial cells.in *P.aeruginosa* has been well studied. Biofilm is an important factor in the quorum sensing mediated virulence in which the bacterial cell encompasses itself with a protective extracellular matrix. Biofilm promotes bacterial infection by resisting antibiotic treatment due to relative impermeability [8].Cell adhesion is an another important quorum sensing mediated virulence factors which initiates the biofilm formation and pathogenicity in the host. Pyocyanin- a secondary metabolite produced by *P.aeruginosa* which can evade the mammalian cells during the time of infection is the major controlled QS mediated virulence factor. Production of various extracellular enzymes such as protease ,elasterase and lipase which facilitates pathogenic invasion is mediated by specific quorum sensing.

Anti qourum sensing agents would offer a way of controlling microbial infections with the advantage of reducing risks of resistance development [9,10,11].The continuing search for new and novel antimicrobials and antipathogenic agents has focused on exploiting the fact that plants surviving in an environment with high bacterial density have been seen to possess protective means against infections [12].Using this argument, researchers are increasingly looking at herbal products in the quest for new therapeutic and antipathogenic agents which might be nontoxic inhibitors of quorum sensing, thus controlling infections without encouraging the appearance of resistant bacterial strains [13].Current literature estimates that 10% of all terrestrial flowering plants on earth have been used by different communities in treating diseases, however, only around 1% have gained recognition and validation [14]. Controlled studies indicate the great potential of phytochemicals to be the richest reservoir of new and novel therapeutics[15]. Although the antimicrobial activities of plant extracts are beyond doubt, in many instances their exact mechanism of antimicrobial functionality is not well understood [16].Searching the literature, it is surprising to find very few works discussing plant extracts and their anti quorum sensing activities. It is believed that plant extracts with well documented antimicrobial activities could possess antipathogenic as well as antivirulent activities, which may not be linked to the growth and inhibition of the microorganism [17,18].The antiquorum sensing activity of herbal plants is very poorly investigated and it is very likely that it will be found that the antimicrobial efficacy is mediated by quorum sensing inhibition. Plants have evolved to produce antiquorum sensing compounds that can be used to quench the quorum sensing signals in the pathogens [19]. In the present study, anti quorum sensing activity of crude ethanolic extract of medicinal plants against quorum sensing mediated virulence factors twisting motility, biofilm formation and cell adhesion, pyocyanin production, proteolytic enzyme activity of *Pseudomonas aeruginosa* has been carried out.

MATERIALS AND METHODS

Collection of plant materials

Rhizome of *Curcuma longa*, leaves of *Ocium tenuiflorum*, *Aegle marmelos*, *Eucalyptus globules*, *Azadirachta indica*, *Cynodon dactylon*, were collected from Agriculuture college and research institute Madurai.

Preparation of Plant Extract

The collected plant material were air-dried under shade at room temperature, finely ground into powder using domestic mixture and stored in an airtight plastic sampling bags for further studies. Extraction was carried out by the modified method of Hussaini and Mahasneh [20].The plant materials were separately extracted twice at room temperature with ethanol 95%(100 mL/10 g of plant material each run). The final ethanol extract of each plant part was filtered using (Whatman No.1) filter paper and evaporated under vacuum at 40 °C using a rotary vacuum evaporator, the concentrated extract thus obtained was collected in screwcap vial and used for further studies.

GC-MS analysis

GC-MS analysis of ethanol extract of concentrated plant extracts was performed using a Perkin-Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS).

Evaluation of anti quorum sensing activity of medicinal plant extracts against Quorum sensing mediated virulence factors.

Bacterial strain and growth condition

Pseudomonas aerugnosa was obtained from Microbial Type culture collection (MTCC), Chandigurh, India and the strain was maintained on tryptic soy agar (TSA)slant. Tryptic soy broth (Hi media) was used for inocula preparation of the bacterial strain. Cultures was inoculated from fresh slopes and incubated with shaking at 37 °C for 24 hours. The following quorum sensing mediated virulence factors such as twitching motility, biofilm production, pyocyanin production and proteolytic activity of *Pseudomonas aeruginosa*

Twitching Motility

500µl of respective plant extracts (final concentration 0.05mg/ml) and 250µl of bacterial inoculums thus prepared in LB broth was mixed in sterile eppendorf tube, kept in room temperature for 30minutes.The respective plant extracts treated cultures were stab inoculated through LB agar plates. The plates were incubated at 32°C for 24hours.Bacterial grow at the interface between the plastic surface and the agar is indicative of twitching motility [21]. To visualize the bacterial growth on the plastic surface, the agar was removed and the plate was stained with a 1% solution of crystal violet [22]. Twitching motility was determined by measuring the diameter of the stained growth.

Biofilm Inhibition

Biofilm inhibition study was carried out by modified method of micro titre plate crystal violet calorimetric assay. In this method nitrocellulose membrane filter was used. A sterile filter(10mm) was transferred to a sterile petriplate containing a layer of cotton moisture with water 250µl of inoculum and 500µl of respective plant extracts were added to the membrane filter paper(Difco, USA).The inoculated filter paper was incubated at 37^{0}C for 72 hours.After incubation, 100µl of 1% v/v aqueous solution of crystal violet was added to the filter paper and is incubated for 30minutes.The dye was removed and the filter paper were washed using sterile distilled water followed by using 95% ethanol and incubated for 15minutes. Biofilm inhibition was studied by determination of the absorbance of the ethanol solubilised mixture at 540 nm in an UV spectrophotometer. Control (without bacteria only crystal violet),three replicates were maintained for each treatment The absorbance of the reaction mixture was read in spectrophotometer at 570nm.

Pyocyanin Production

Effect of plant extracts on pyocyanine production was done by the modified method of Duan et al [23]. Pyocyanine was extracted from the supernatant fraction of *Pseudomonas aeruginosa* grown in trypticase soy broth medium with 500µl of plant extracts for 24hours. 5ml sample of the supernatant was mixed with 5ml chloroform and the lower organic layer was separated. To this layer 1.5ml of 0.2M of HCl was added and the pyocyanine rich organic layer was separated. The amount of the pyocyanine within the extracted layer was determined by measuring the absorbance at 520nm.

Cell Adhesion

Cell adhesion was studied by using 96 well flat bottom micro well plate previously coated with bovine serum albumin (BSA). Wells were coated with 150µl of freshly prepared 1.0% BSA, incubated at 30 ^{o} C for 30 minutes. After the incubation period, wells were washed thrice with sterile phosphate buffered saline (PBS).50 µl of bacterial inocula thus prepared was transferred to the well followed by the addition of 50µl of the respective plant extracts. Seeded microtitre plate was incubated at 37°C for 24 hours. Cells were allowed to adhere and the non-adhered cells were washed 5 times with PBS at room temperature. Adhered cells were detected by adding 50µl of 0.1% crystal violet per well, incubated at room temperature for 30 minutes. Wells were washed with sterile distilled water to remove excess stain. 10µl of ethanol was added to fix the adhered cells.50µl of 0.2% Triton X was added to the wells for the lyse of cells and the absorbance was read at 570nm.

Proteolytic activity

Proteolytic activity was carried out by modified method of Lowry's et al [24].

Crude enzyme preparation

0.1 ml of tryptic soy broth bacterial culture was inoculated into 100 ml of protease production media (Yeast extract-5mg/l,Peptone-10mg/l,Glucose-10mg/l,Caesin-15mg/L) supplemented with 200µl of respective plant extracts.Flasks were incubated at 37^{o}C for 48 hours. Broth was centrifuged after the incubation period at 10,000rpm for 10 minutes, the collected supernatant was used as the source of protease enzyme.

Enzyme Activity

Enzyme activity was assayed using casein as the substrate. The reaction mixture consisted of 0.25 ml of 50mM sodium phosphate buffer (pH 7.0) containing 2.0% (w/v) of azocasein and 0.15 ml of enzyme solution. After incubating at 25ºC for 15 min, the reaction was stopped by adding 1.2 ml of 10.0% (w/v) TCA, incubating at room temperature for an additional 15 min, and then the precipitate was removed by centrifugation at 8,000 ´ g for 5min. 1.4ml of 1.0M NaOH was added to 1.2 ml of the supernatant, and its absorbance was measured at 440nm.

RESULT AND DISCUSSION

Quorum sensing is a system of stimulus and response correlated to population density. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of their local population [24,25]. The deeper understanding of microbial cell communication promises to shed light on the complexities of the host-microbe relationship and may lead to novel therapeutic applications The researchers have made a step up to manage the infections.One of the greatest accomplishments of modern medicine has been the development of antimicrobial pharmaceuticals for the treatment of infectious diseases by the inhibition of quorum sensing [26].

In the present study, anti quorum sensing activities of Rhizome of *Curcuma longa*, leaves of *Ocium tenuiflorum*, *Aegle marmelos*, *Eucalyptus globules*, *Azadirachta indica*, *Cynodon dactylon* has been studied against clinical isolate of *P.aeruginosa* has been carried out.In the present investigation ,solvent extraction and bioassays have led to the identification of potential compounds with anti quorum sensing activity against *Pseudomonas aeruginosa*. GC-MS was carried out to characterize the bioactive compounds. The name, molecular weight and structure of the components of the test materials were ascertained. The various phytochemicals which contribute to the medicinal activity of the plant are listed in Table 1 to 6.

GC-MS analysis of the ethanol extract of *Cynodon dactylon* and *Eucalyptus globulus* revealed eight (Figure 1) and eleven major peaks (Figure 2) and the retention time for the major peaks was in the range of 16.8 to 21.65 and 11.6 to 25.93 respectively. The peaks constituted 36.8 to 100 %.and 18.3 to 100%. Nine (figure 3) and two (figure 4) major peaks was recorded in *Azadirachta indica* and *Ocium tenuiflorum* extracts with the retention time range of 16.9 to 25.6 (57.0 to 100.0 % constitution and 4.7 to 8.97 % (5.6 and 4.4 % of constitution respectively. Rhizome extract of C*urcuma long* (Figure 5) and leaf extract of *Aegle marmelos* (Figure 6) revealed nine major peaks of 12.17 to 19.2(78.4 to 100 % constitution) and 15.25 to 24.42 retention time (50 to 100 % constitution. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 65,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library which revealed the presence of various constituents.

Table 1.Percent composition (%) of constituents in the ethanolic extract of *Cynodon dactylon*

Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
2-Penta,6,10,14-trimethyl	16.38	$C_{18}H_{36}O$	262.43	66.2%
1-Dodecanol,3,7,1 1-trimethyl	17.18	$C_{15}H_{32}O$	382.29	36.8%
Hexadecanoic acid- ethyl ester	17.98	$C_{18}H_{36}O_2$	284.5	49.6%
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.17	$C_{20}H_{40}O$	296.43	100%
Ethyl Oleate	19.65	$C_{20}H_{40}O$	310.51	100%
Heptadecanoic acid 15-methyl-ethyl ester	19.85	$C_{20}H_{40}O_2$	326.55698	100%
Eichosanoic acid- ethyl ester	21.65	$C_{21}H_{42}O_2$	326.5570	74.1%

Table 2. Percent composition (%) of constituents in the ethanolic extract of of *Eucalyptus globulus*

	Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
	Patchoulene	11.6	$C_{15}H_{24}$	204.35106	18.3%
	Globulol	13.57	$C_{15}H_{26}O$	222.36	37.1%
	a-phellandrene	15.9	$C_{10}H_{16}$	136.23404	100%
	Pentadecanoic acid,14-methyl-methyl ester	17.23	$C_{17}H_{34}O_3$	286.45	100%
	1,2-benzenedicarboxilic acid, butyl octy ester	17.72	$C_{20}H_{30}O_4$	334.4498	100%
	8,11-Octadecadienoic acid, methyl ester	18.92	$C_{19}H_{34}O_2$	294.4721	100%
	Ethanol,2-(9-octadecenyloxy)-,(z)-	19.15	$C_{20}H_{40}O_2$	312.534	88.1%
	Oleic acid	19.92	$C_{18}H_{34}O_2$	282.461360	100%
9.	2,3-Dihydroxypropyl elidate	22.03	$C_{21}H_{40}O_4$	356.5399	57.3
10.	Hexadeconoic acid,1-(hydroxymethyl).1,2-ethannediyl ester	23.18	$C_{35}H_{68}O_5$	568.91	39.1%
11.	9-Octadecenoic acid(z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	25.93	$C_{18}H_{34}O_2$	282.461360	76.8%

Anti quorum sensing activity against Virulence factors of *Pseudomonas aeruginosa*

Table 3. Percent composition (%) of constituents in the ethanolic extract of of *Azadirachta indica*

Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
Pentadecanoic acid,14-methyl,methyl ester	16.9	$C_{17}H_{34}O_2$	270.450660	100%
Hexadecanoic acid, ethyl ester	17.58	$C_{18}H_{36}O_2$	284.5	76.8%
8,11-Octadecadienoic acid, methyl ester	18.58	$C_{19}H_{34}O_2$	294.4721	100%
10- Octadecadienoic acid, methyl ester	18.63	$C_{19}H_{36}O_2$	296.48794	100%
Ethanol,2.(9.octadececenyloxy).(z)	18.82	C24H47 N O3	399.65076	100%
Oleic acid	19.65	$C_{18}H_{34}O_2$	282.461360	100%
9-Octadecenoic acid(z)2-hydroxy 1-(hydroxymethyl)ethyl ester	21.68	$C_{18}H_{34}O_2$	282.461360	37.3%
Hexadecanoic acid,1-(hydroxymethyl)1-2 ethanediyl ester	22.87	$C_{35}H_{68}O_5$	568.91	33.7%
9-Octadecenoic acid(z)2-hydroxy 1-(hydroxymethyl)ethyl ester	25.6	$C_{18}H_{34}O_2$	282.461360	57%

Table 4. Percent composition (%) of constituents in the ethanolic extract of of *Ocium tenuiflorum*

Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
9-Octadecene,1,1-(1,2-ethanediylbis(oxy))bis-,(ZZ).	4.7	$C_{22}H_{46}O_4$	310.6027	5.6%
Ethyl 9,9-diformylnona-2,4,6,8-tetraenoate	8.97	$C_{13}H_{14}O_4$	234.2491	4.4%

Table 5. Percent composition (%) of constituents in the ethanolic extract of of C*urcuma long*

Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
Benzene,1-methyl-4-(1,2,2-trimethylcyclopentyl)-,(R)	12.17	$C_{15}H_{22}$	202.3352	100%
6-(p.Toly)-2-methyl-2-heptenol	13.72	C11H14O	162.231	100%
6-(p-Toly)-2-methyl-2-heptenol	14.7	C11H14O	162.231	100%
7-Oxabicyclo(4.1.0)heptane,1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-(E)	15.43	$C_{15}H_{24}O$	220.3519	100%
Acetic acid,3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	15.75	$C_{17}H_{26}O_3$	278.3882	100%
7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]loctane	16.08	C38H64O3	568.9165	100%
10-Octadecenoic acid, methyl ester	18.97	$C_{19}H_{36}O_2$	296.4879	100%
Heptadecanoic acid, 16-methyl-,methyl ester	19.2	$C_{19}H_{38}O_2$	298.5038	78.4%

Table 6. Percent composition (%) of constituents in the ethanolic extract of of *Aegle marmelos*

S.no	Name of the compound	Rt	Molecular Formula	Molecular Weight	Peak area %
1.	Pentadecanoic acid, 14-methyl-, methyl ester	15.25	$C_{17}H_{34}O_2$	270.450660	100%
2.	Hexadecanoic acid, ethyl ester	15.95	$C_{18}H_{36}O_2$	284.5	100%
3.	l-[+]Ascorbic acid 2,6-dihexadecanoate	16.35	$C_{38}H_{68}O_8$	652.9417	100%
4.	Oleic acid	17.68	$C_{18}H_{34}O_2$	282.46136	100%
5.	9-Octadecenoic,(E)-	18.18	$C_{18}H_{34}O_2$	282.46136	100%
6.	9-Octadecenoic,(E)-	20.22	$C_{18}H_{34}O_2$	282.46136	96.7%
7.	9-Octadecenoic,(E)-	20.77	$C_{18}H_{34}O_2$	282.46136	100%
8.	Hexadecanoic acid,2,3-dihydroxypropyl ester,(n)-	21.4	$C_{19}H_{38}O_4$	330.50262	100%
9.	9-Octadecenoic acid(Z)-,2-hydroxy-1.(hydroxymethyl)ethyl ester	24.42	$C_{18}H_{34}O_2$	282.461360	50%

Anti quorum sensing activity of the plant extracts was evaluated against twitching motility, biofilm production, pyocyanin production and proteolytic activity. Twitching motility is associated with high cell densities and cell-cell communication, which are the hallmarks of quorum-sensing systems in bacteria. Recently, quorum sensing has been shown to be involved in initiating and controlling motility [27]. Diameter of the stained growth (radius) of tested bacterial strain treated with the plant extracts was found to be less that of control (Figure 7).Among the plant extracts tested, *Eucalyptus globules* revealed complete inhibition with 0 cm of stained growth followed by *Aegle marmelos* (1.0 cm) and *Cynodon dactylon* (1.5cm).But twitching motility did not seem to affected significantly by *Ocium tenuiflorum* treatment (3.0 cm) and *Curcuma longa* (2.8cm).

To analyse the anti quorum sensing activity of plant extracts on *Pseudomonas aeruginosa*, biofilm formation and cell adhesion were investigated. Biofilm an another important QS mediated virulence factor is now being considered as the major target for the development of therapeutic agents against the pathogenic microorganism. Biofilm promotes bacterial infection by resisting antibiotic treatment due to relative impermeability [28].Inhibitory effect of plant extracts against biofilm of *Pseudomonas aeruginosa* was carried out by microtitre plate crystal violet spectrophotometric assay. Figure 8 shows effect of plant extracts on biofilm formation which revealed all the tested plant extracts reduced significantly. *Eucalyptus globules* and *Aegle marmelos* were reduced the biofilm formation by 99.77 and 99.05% .97.92,97.47 and 95.26% of reduction was recorded in *Ocimum tenuiflorum, Cynodon dactylon* and *Azadirachta indica*. Besides biofilm formation effect of plant extracts on cell adhesion was also studied. Cell adhesion initiates the biofilm formation and pathogenicity in the host All the tested plant extracts

showed similar inhibitory effect (Figure 9). We also studied the effect of plant extracts on the ability to reduce the pyocyanin production. Results of pyocyanin production reduction was presented in figure 10. Pyocyanin pigment production was reduced to 51.85 % in *Aegle marmelos* and *Azadirachta indica* (25.46%) which showed high rate of reduction when compared to *Cynodon dactylon* (19.35%), *Eucalyptus globules*(5.46%) and *Ocimum tenuiflorum* (5.25%).

Figure 1.GC-MS chromatogram of *Cynodon dactylon*

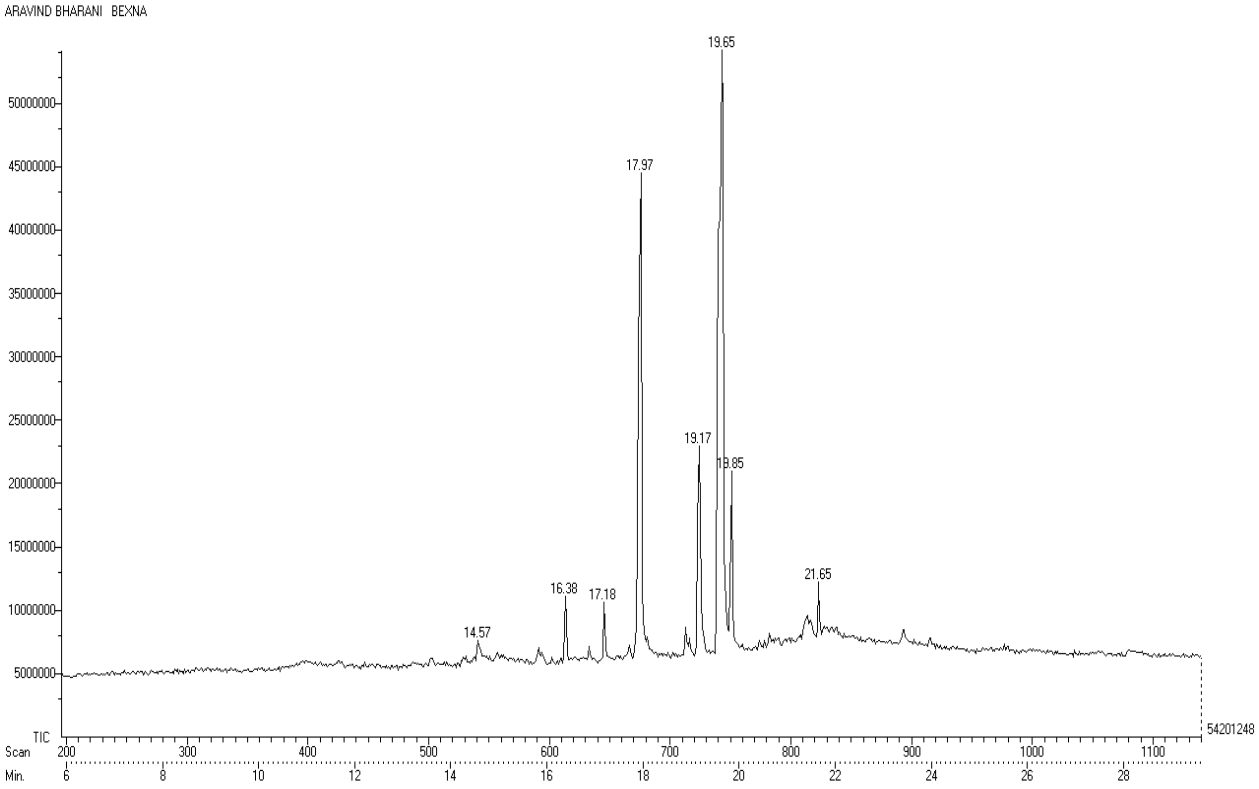


Figure 2.-GC-MS Chromatogram of ethanolic extract of *Eucalyptus globulus*

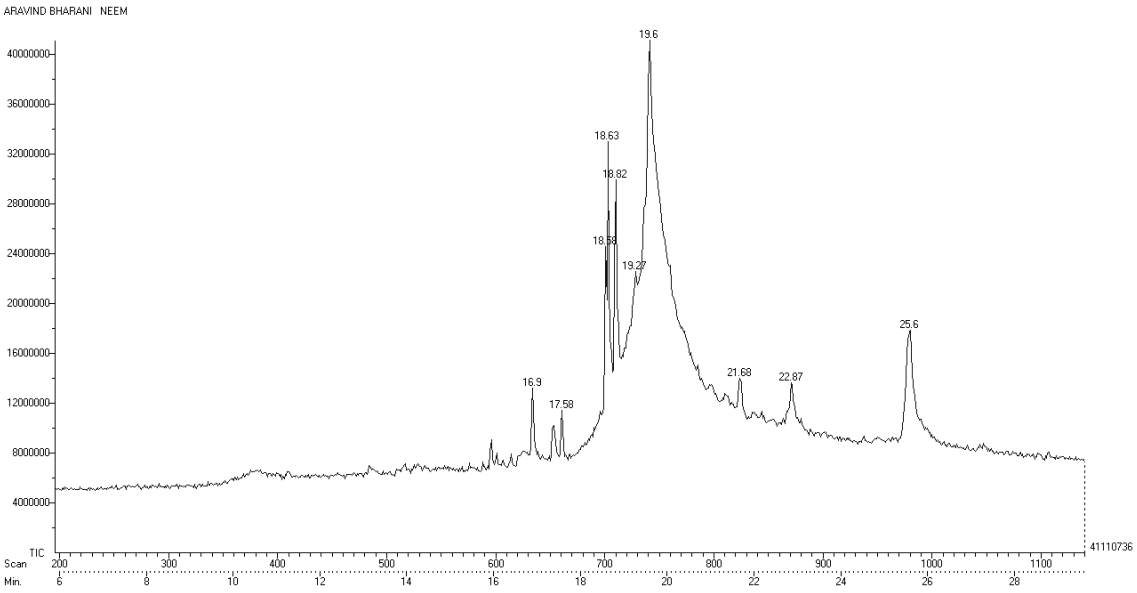


Figure 3.GC-MS Chromatogram of ethanolic extract of *Azadirachta indica*

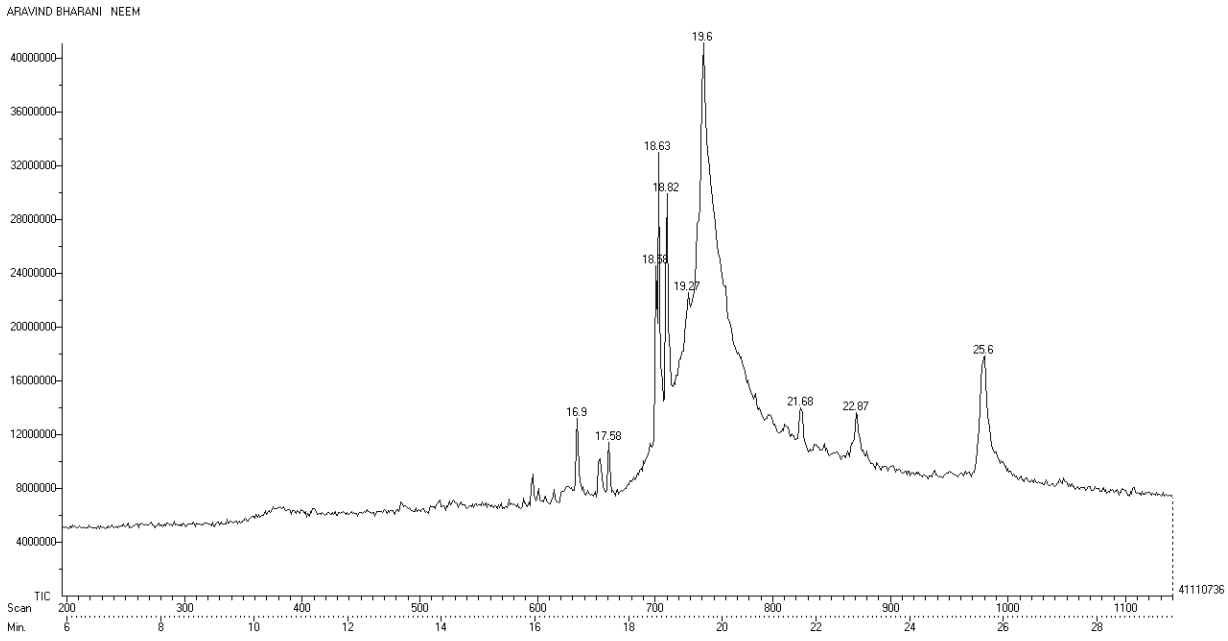
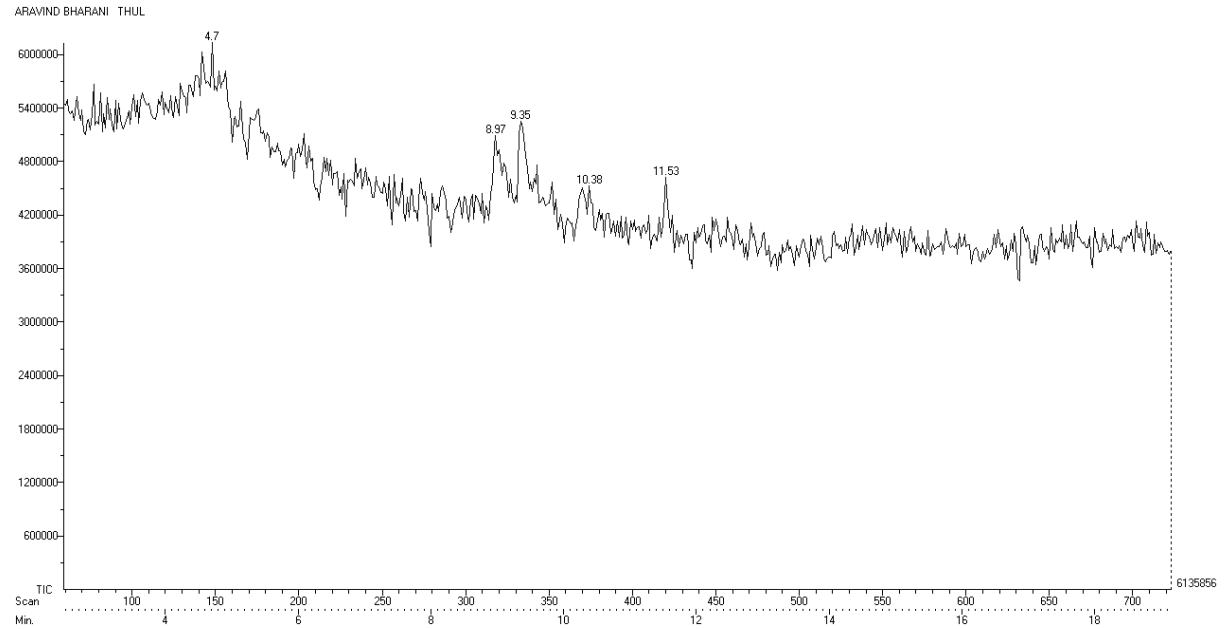


Figure 4.GC-MS Chromatogram of ethanolic extract of *Ocimum tenuiflorum*



Total proteolytic activity of *P.aeruginosa* treated with plant extracts was determined by measuring the reduction of azocasein as the substrate by the crude protease present in the supernatant (Figure 11)..Maximum reduction of total protease activity was observed in *Eucalyptus globules* (2.5 units/ml), *Aegle marmelos* (11.7 units/ml and Cynodon dactylon (20.1 units/ml) Least reduction of protease activity was observed in *Azadirachta indica* (49.5 units/ml) and *Ocimum tenuiflorum* (47.5 %)

The mechanism of anti quorum sensing activity appeared to be a net effect of the ability of phytochemicals in the plant extracts to interfere with the activity of acyl homoserine lactone (AHL) a signalling molecule controls quorum sensing activities. AHL signalling molecules are highly conserved as they have the same homoserine lactone moiety, but differ in the length and structure of the acyl side chain. The *N*-acylated side chain consists of fatty acids that vary in chain length (ranging from 4–18 carbons), degree of saturation, and the presence of a hydroxyl -oxo- or no substituent at the C3 position. AHL molecules are synthesized by LuxI synthase using *S*-adenosyl methionine and an intermediate of fatty acid biosynthesis as substrates. The generated AHL molecules will then bind to LuxR protein as a receptor of AHL followed by subsequent regulation of downstream gene expressions. Each of the LuxR-type

proteins is highly selective for its cognate AHL signal molecules [29]. Phytochemicals are modulate the bacterial synthesis of AHL and inturn inhibit QS. Many natural extracts are believed to inhibit QS by either interfering with AHL activity by competing with them due to their structural similarity and/or to accelerate the degradation of the LuxR/LasR receptors for the AHL molecules. Inhibition of quorum sensing mediated virulence factors of *P.aeruginosa* by bud extract of Clove (*Syzygium Aromaticum*) has been reported [18,30]. Presence of potential phytochemicals in the plant extracts may degraded acyl homoserine lactone (AHL) and inhibited QS mediated virulence factors of *P. aeruginosa*. Plant materials used in the present study are traditional medicinal plants and widely used worldwide. They have not been cause any adverse side effects. Hence, they should be regarded as safe.it has not been shown to exhibit adverse effects. Hence, it should be regarded as safe. However, for future work on clinical trials or application, toxicity and potential adverse effects must first be ruled out. It is suggested that cytotoxicity test should be carried out to ascertain the safety of the compounds. Taken these results together, the tested plant extracts are shown to possess active compounds that can significantly inhibit various virulence determinants in human pathogen *P. aeruginosa*

Figure-5-GC-MS Chromatogram of ethanolic extract of *Curcuma longa*

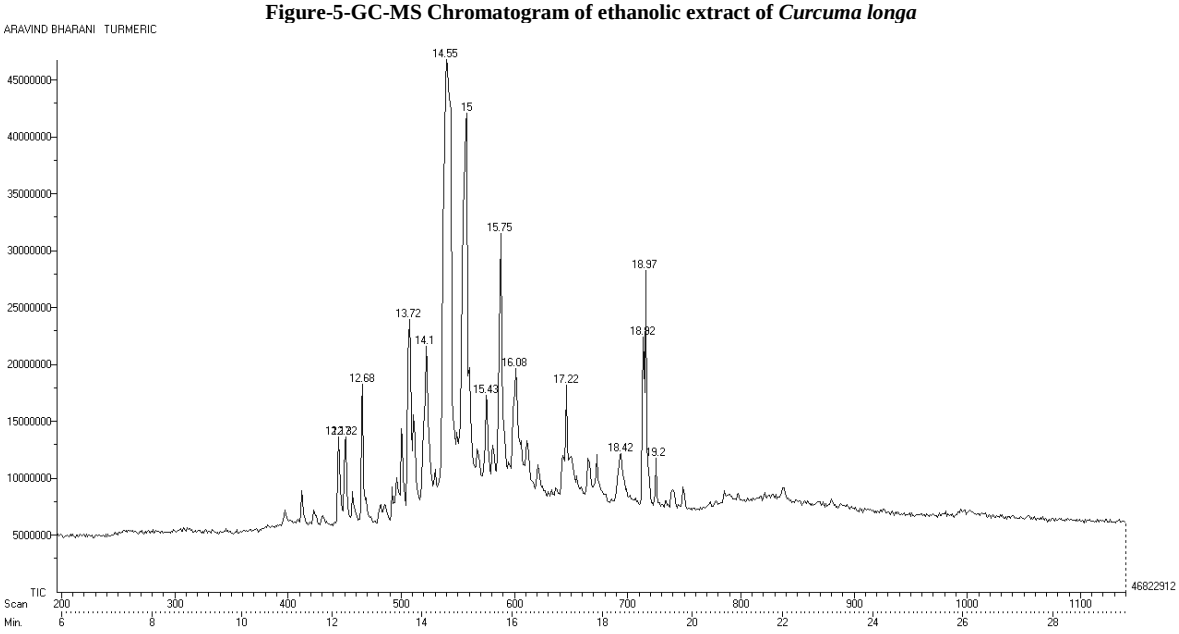


Figure 6. GC-MS Chromatogram of ethanolic extract of *Aegle marmelos*

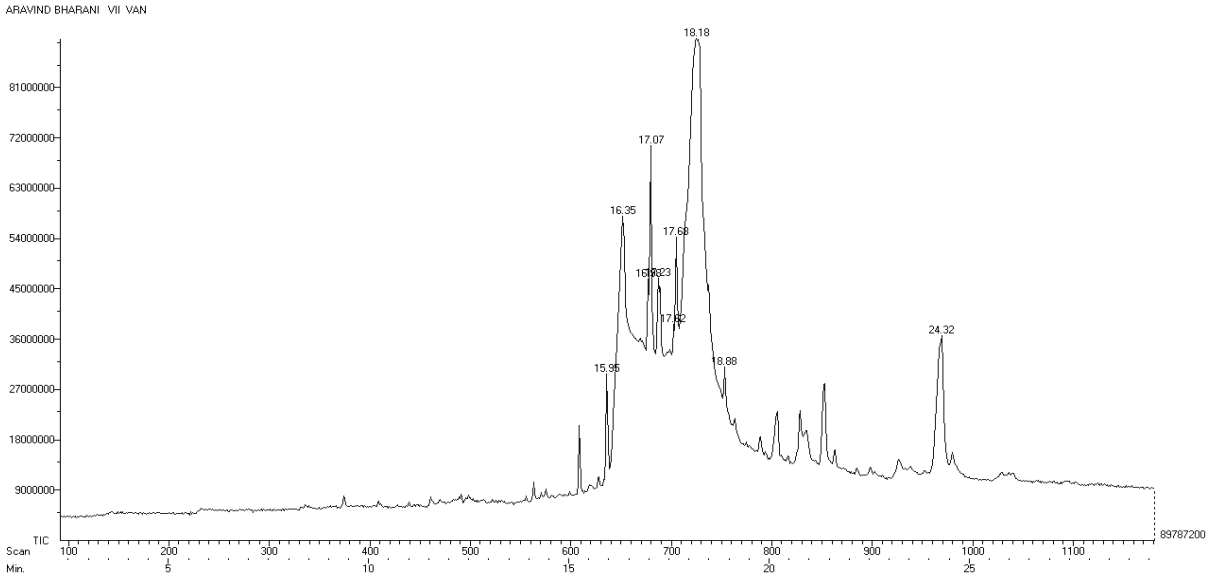


Figure 7..Effect of ethanolic extracts of medicinal planst against twitching motility of *Pseudomonas aeruginosa*

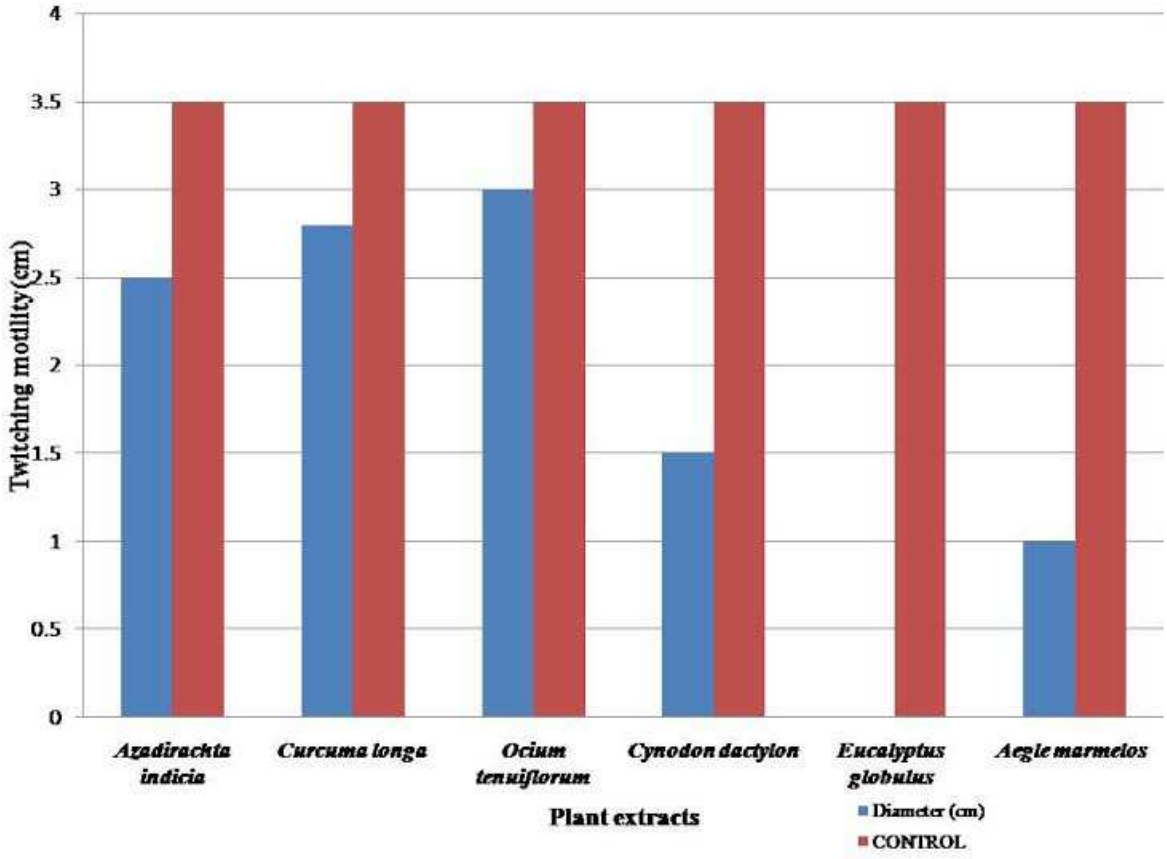


Figure 8. Effect of ethanolic extracts of medicinal plants against biofilm inhibition of *Pseudomonas aeruginosa*

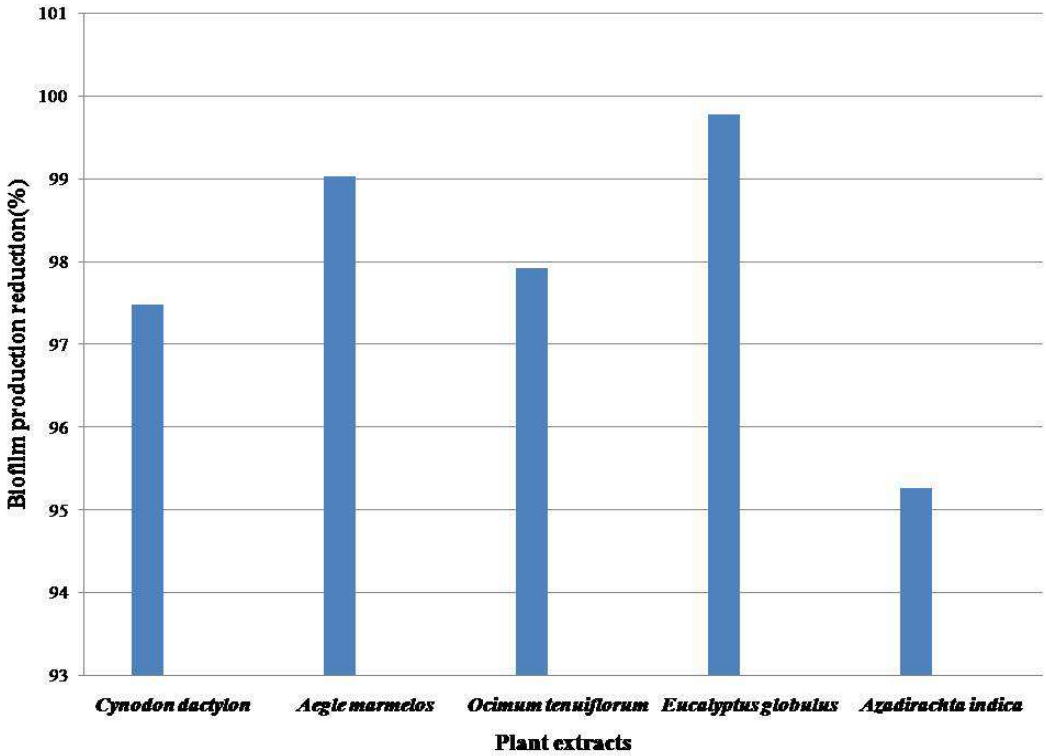


Figure 9: Effect of ethanolic extracts of medicinal plants against Cell adhesion inhibition of *Pseudomonas aeruginosa*

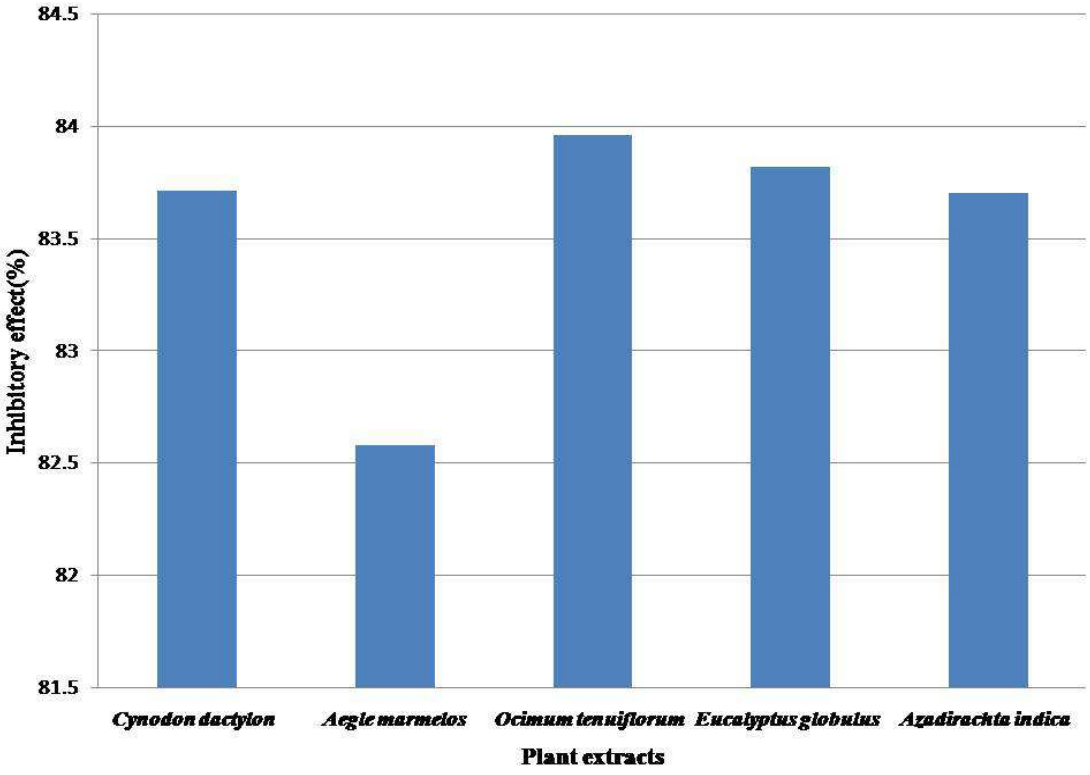


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Figure 10: Effect of ethanolic extracts of medicinal plants against Pyocyann production reduction of *Pseudomonas aeruginosa*

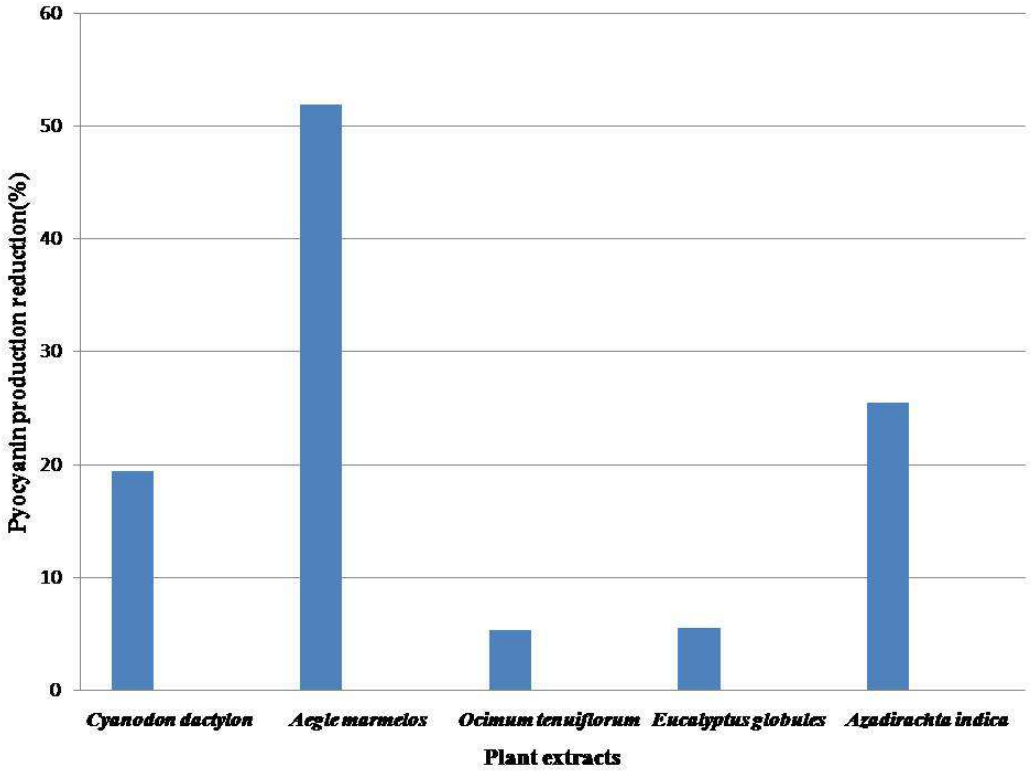
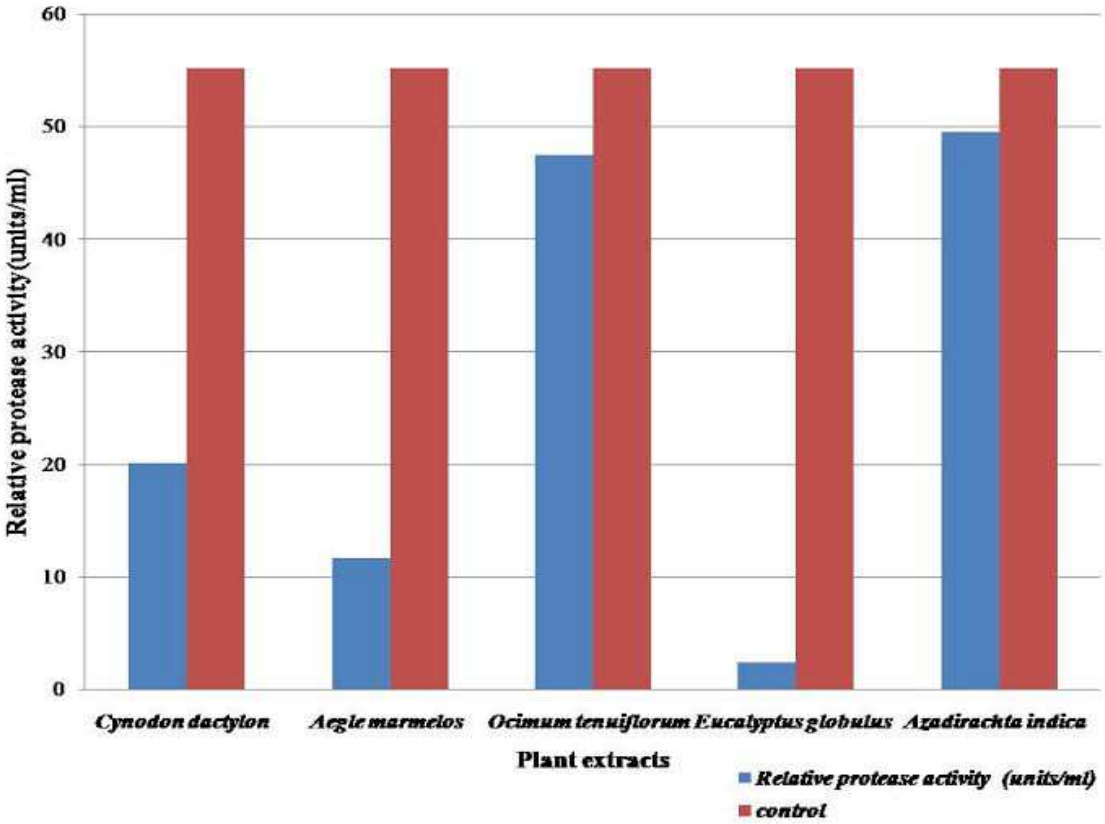


Figure 11: Effect of ethanolic extracts of medicinal plants against total proteolytic activity of *Pseudomonas aeruginosa*



In conclusion, continuous emergence of multidrug-resistant bacteria caused increased need of anti-pathogenic and anti-infective strategy to combat bacterial infections. Natural products provide alternative medicine for treating emerging bacterial infections without leading to antibiotic resistance. Previous studies have shown anti-QS activity in plants. The presence of active compounds exhibiting anti-QS activity in the plant extracts maybe useful for the development of anti-infective drugs. Our laboratory is currently elucidating the chemical structure of these active compounds to understand the anti-QS mechanism in QS bacteria.

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